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Discovery of new anti-inflammatory agents*

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Abstract: We have synthesized a series of novel hydrazines and hydrazino alcohols that specifically inhibit vascular adhesion protein-1 (VAP-1), a human endothelial cell adhesion molecule with a well-documented role in inflammation. VAP-1 is a semicarbazide-sensitive amine oxidase (SSAO), and the enzyme activity has been demonstrated to have a role in VAP-1 function. An indane hydrazino alcohol was able to reduce clinical symptoms of inflammation in experimental arthritis in rodents and has the potential to be a novel anti-inflammatory drug.

INTRODUCTION

Vascular adhesion protein-1 (VAP-1) is a human endothelial cell adhesion molecule that has several unique properties that distinguish it from the other inflammation-related adhesion molecules. Analysis of VAP-1 amine oxidase activity showed that VAP-1 belongs to the class of membrane-bound monoamine oxidases (MAOs) termed semicarbazide-sensitive amine oxidases (SSAOs). VAP-1/SSAO activity has been proposed to be directly involved in the pathway of leukocyte adhesion to endothelial cells by a novel mechanism that may involve the production of proinflammatory mediators such as hydrogen peroxide and direct interaction of leukocyte ligands with the active site of the enzyme. In human clinical tissue samples, expression of VAP-1 is induced at sites of inflammation [1–3].

Since the inhibition of VAP-1/SSAO activity would offer an attractive target for the development of novel anti-inflammatory drugs, our aim was to find a new, small-molecule inhibitor of this unique enzymatic function. The VAP-1/SSAO inhibitory activity was determined in a similar way to the MAO inhibitory activity by a standard in vitro biochemical method [4].

LEAD SELECTION

A ca. 2000-membered library with a wide structural diversity, containing mainly 1,2- and 1,3-difunctional compounds and saturated heterocycles, prepared in the recent years in the Institute of Pharmaceutical Chemistry, University of Szeged, was applied to find the first hits showing VAP-1 inhibitory activity. Perhydro-1,3,4-benzoxadiazine derivatives **1** and **2** proved to be the most active compounds in this library (VAP-1 IC_{50} **1**: 3.8 μM, **2**: 0.6 μM) and were chosen as the starting point for further development. Investigation of differently substituted and saturated 1,3,4-oxadiazine derivatives (**3**–**5**) showed that compounds of type **3** exhibit the most pronounced VAP-1/SSAO inhibitory activity (Fig. 1).

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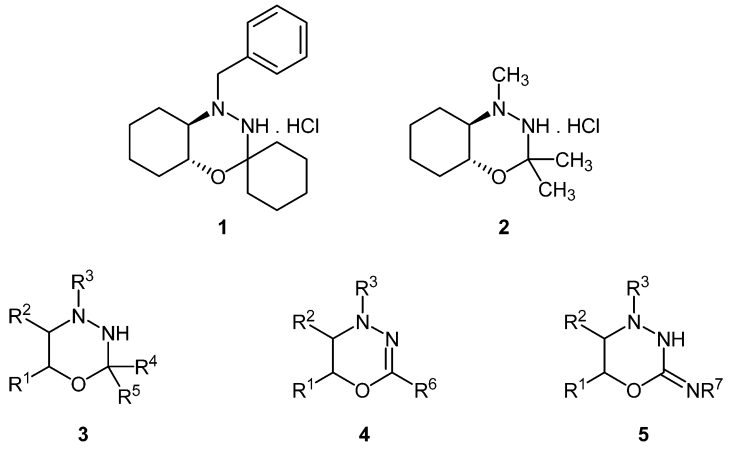
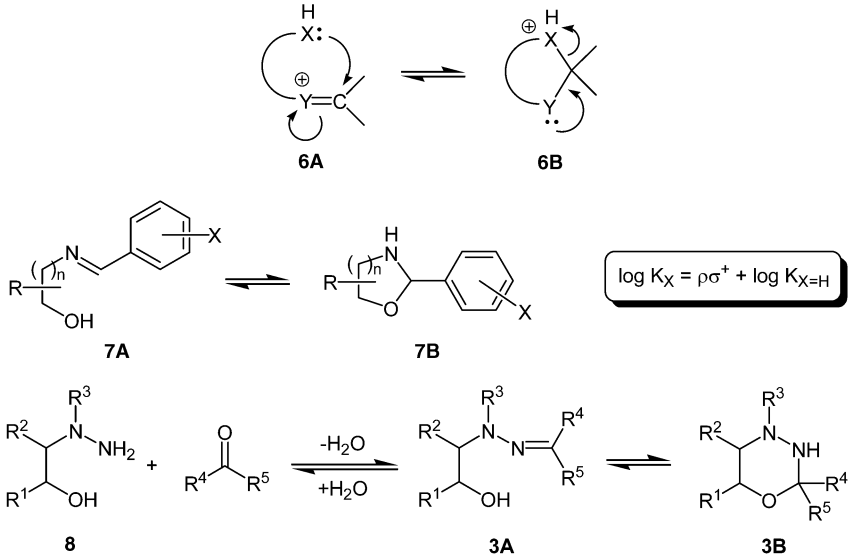


Fig. 1

The structure and reactivity of tetrahydro-1,3,4-oxadiazines (**3**) can be characterized by ring-chain tautomerism [5–7], a well-known phenomenon among the *N*-unsubstituted, five- and six-membered saturated 1,3-*X*,*N*-heterocycles [8,9]. The tautomeric ratios in the equilibria of such compounds (e.g., **7**) are strongly influenced by the steric and electronic parameters of the substituents. According to ^{13}C NMR and X-ray data, compounds **1** and **2** proved to be ring-closed tautomer both in $CDCl_3$ solution and in the solid state. Despite the highly shifted equilibrium, our further studies on compounds **3** led to the conclusion that these compounds are prodrugs: the VAP-1/SSAO inhibitory activity of 1,3,4-oxadiazines is caused by the hydrazino alcohols **8** liberated by the hydrolysis of the open forms (**3A**) of the tautomeric equilibria (Scheme 1). The ring-chain tautomeric prodrug concept is an alternative way for the possible therapeutic application of biologically active amino alcohol or amino thiol derivatives (e.g., ephedrine, cystein) [9,10].

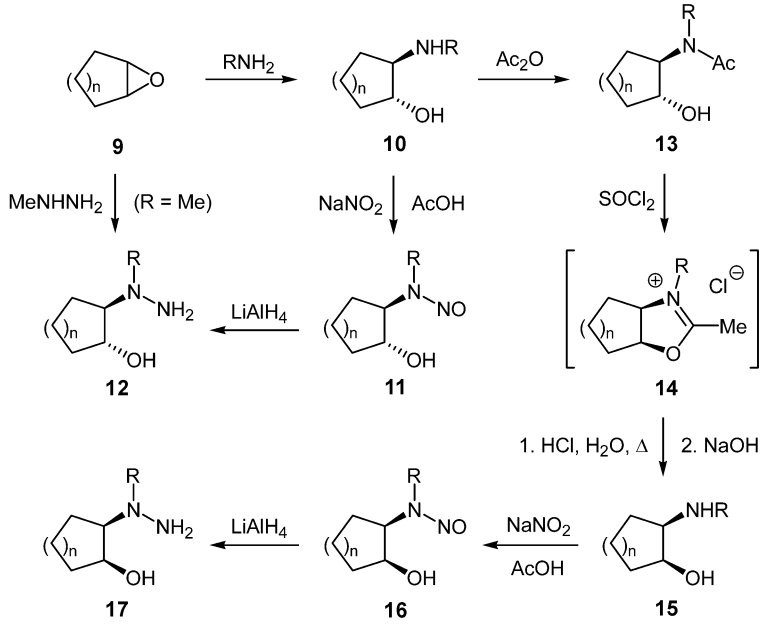


Scheme 1

SYNTHESES

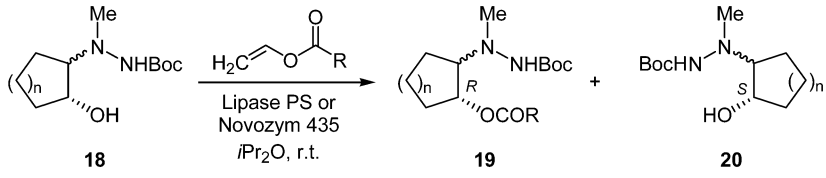
Our further synthetic efforts were concentrated on the substituent pattern of the hydrazino alcohol structure of **8**. Our aim was to prepare a large number of hydrazino alcohol derivatives for the purpose of providing a wide structural diversity to establish structure–activity relationships of the VAP-1/SSAO inhibitory action. In the synthesis of the target hydrazino alcohols, the well-known methods of the preparation of substituted hydrazine derivatives were applied [11–14].

Alicyclic hydrazino alcohols **12** and **17** were prepared from the corresponding epoxides. Ring opening with primary amines resulted in the *trans* amino alcohols **10**, which were converted to the corresponding hydrazino derivatives **7** by *N*-nitrosations and the subsequent $LiAlH_4$ reductions. *N*-Methyl-substituted *trans* hydrazino alcohols (**12**, R = Me) could also be obtained directly from the epoxides **9** by ring openings with methyl hydrazine. Ring-closures and hydrolysis of the *N*-acetyl derivatives of the *trans* amino alcohols (**13**) gave the *cis* counterparts **17** by inversion, which were transformed to the *cis* hydrazino alcohols by using the above method (Scheme 2).



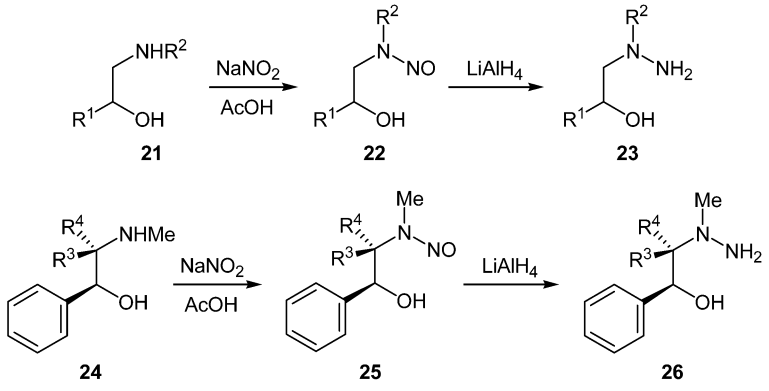
Scheme 2

Enantiomers of the *N*-methyl-substituted, five- and six-membered *cis* and *trans* alicyclic hydrazino alcohols were also prepared by using selective acylation of the N-Boc derivatives (**18**) with vinyl acetate or butyrate in diisopropyl ether in the presence of lipase enzymes. Similarly to other cycloalkanols, enzymatic acylation of **18** exhibited R-selectivity (Scheme 3) [15].



Scheme 3

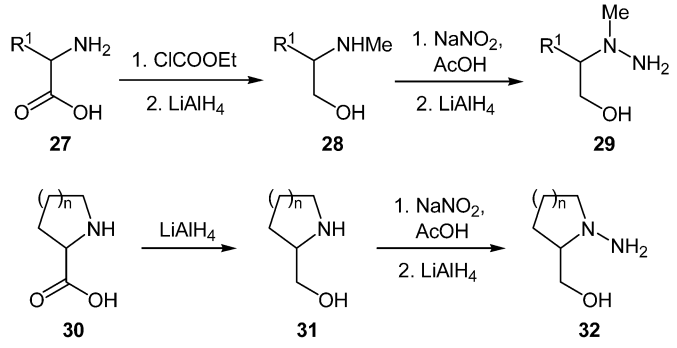
Hydrazino derivatives of some *C*-1- and *N*-substituted aliphatic 1,2-amino alcohols **21** were also synthesized by using the method applied in the case of alicyclic analogs. The *N*-amino-substituted (±)-ephedrine and some of its *C*-substituted homologs (**26**) were prepared according to the literature procedures [16–18] applied earlier for the synthesis of *N*-aminoephedrine and *N*-aminopseudoephedrine derivatives (Scheme 4).



R^1 = H, Me; R^2 = Me, CH_2Ph; R^3 = Me, Et, Pr; R^4 = H, Me

Scheme 4

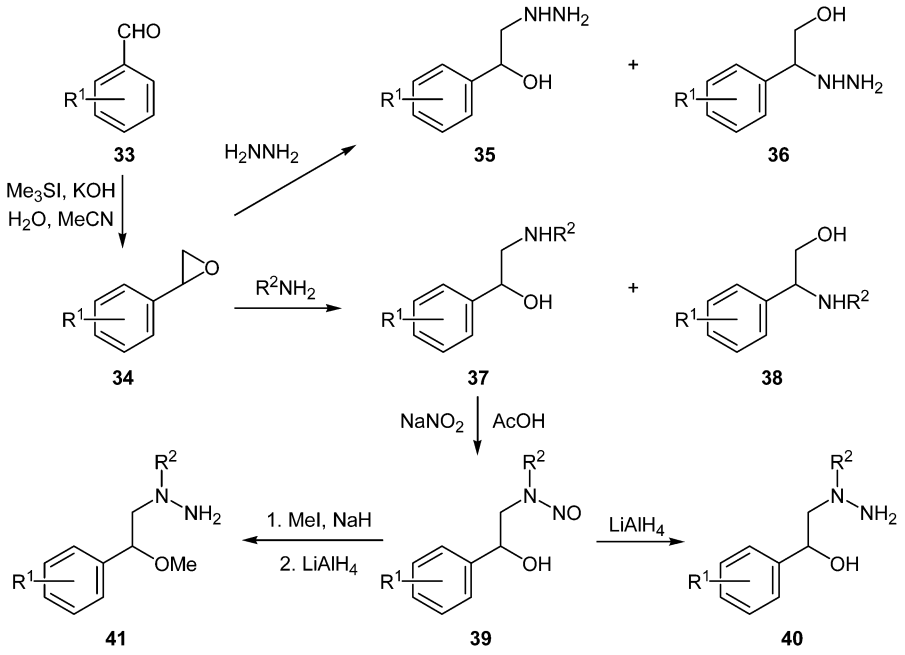
By transformations of racemic acyclic or cyclic α-amino acids (**27** and **30**) *C*-2- and *N*-substituted 1,2-amino alcohols (**28** and **31**) were obtained which were converted to the corresponding hydrazine derivatives by the usual manner [12,13] (Scheme 5).



R^1 = *i*Pr, Ph, CH_2Ph; n = 1, 2

Scheme 5

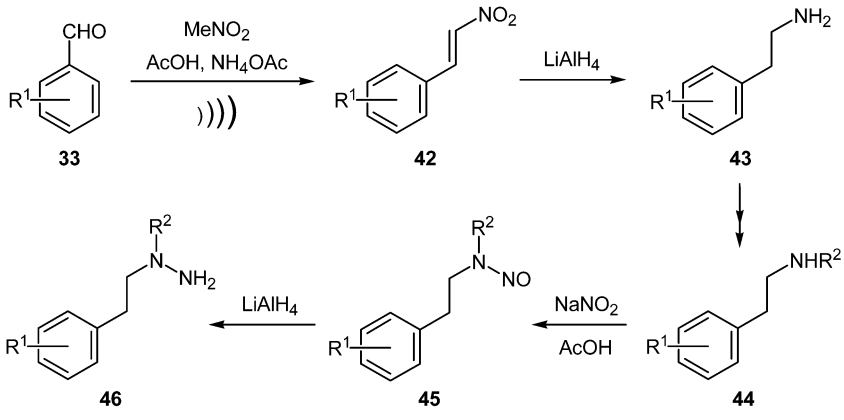
Aryl-substituted hydrazino alcohols **40** were also synthesized by the usual nitrosation/reduction reactions starting from the corresponding amino alcohols **37**, which were prepared by ring-opening reactions of the corresponding styrene oxides **34** with primary amines. These epoxide openings resulted in mixtures of the regioisomeric amino alcohols (**37** and **38**) from which the *major* diastereomer (**37**) was isolated in each case by crystallization. Ring-opening of styrene oxides **34** with hydrazine hydrate again gave mixtures of regioisomeric hydrazino alcohols (**35**:**36** = ca. 2:1) [19], which were separated by fractional crystallization of the maleate salts in the case of the phenyl derivative (R^1 = H). Reaction sequences for the preparation of SAMP/RAMP [20] were applied for the *N*-nitroso intermediates **39**: *O*-methylations and the subsequent $LiAlH_4$ reductions resulted in the corresponding hydrazino alcohol *O*-methyl ethers **41** (Scheme 6).



R^1 = H, Me, Cl, F, OMe; R^2 = alkyl, aralkyl

Scheme 6

To test the lack of the hydroxyl group on the VAP-1/SSAO inhibitory activity, some 2-arylethylhydrazines (**46**) were prepared from the corresponding 2-arylethylamines (**44**) by *N*-nitrosations and $LiAlH_4$ reductions. Compounds **44** were obtained by usual transformations starting from the nitrostyrene derivatives (**42**) of aromatic aldehydes [21] (Scheme 7).

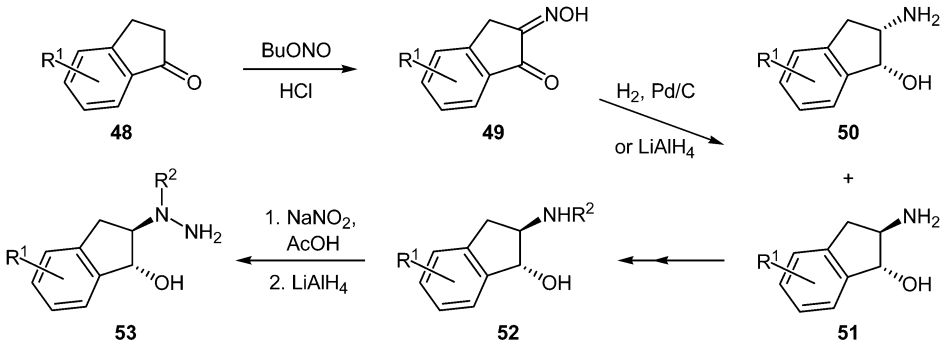


R^1 = H, Me, Cl, F, OMe; R^2 = alkyl, aralkyl

Scheme 7

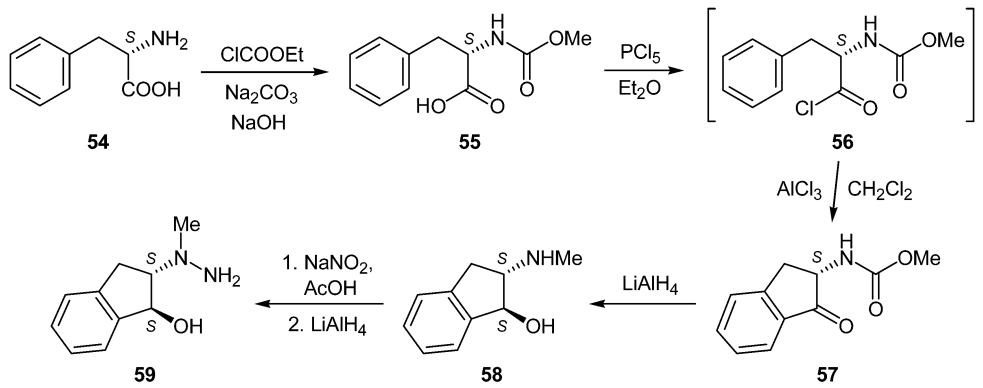
The *trans* indane hydrazino alcohols (**53**) can be regarded as conformationally restricted pseudoephedrine derivatives. Compounds **53** were prepared from the corresponding indane amino alcohols **51** obtained by the catalytic reductions of the isonitrozo ketones **49** [22,23]. From the *cis-trans* isomeric

mixture formed in this latter reduction, the *major trans* isomer (**51**) could be easily isolated by crystallization (Scheme 8).



Scheme 8

The preparation of enantiomerically pure indane hydrazino alcohols was also achieved starting from the easily available indane amino alcohol enantiomers [24]. The *S*,*S*-amino alcohol (**58**) was obtained starting from L-phenylalanine (**54**) which was converted to the *S*,*S*-hydrazino alcohol (**59**) by the usual method (Scheme 9).



Scheme 9

STRUCTURE–ACTIVITY RELATIONSHIPS

On the basis of the VAP-1/SSAO inhibitory effects of the prepared hydrazines and hydrazino alcohols [25–27], some general remarks concerning the structure–activity relationship could be deduced. An aryl substituent at position of 2-hydrazinoethanol increased the VAP-1/SSAO inhibitory activity. Substituents on the aromatic ring did not prove to have great influence on the inhibitory activity. The lack of the hydroxyl group did not decrease the VAP-1/SSAO inhibitory effect, however, the VAP-1/SSAO selectivity over MAO-A and B inhibition was significantly decreased. Aralkyl or bulky alkyl substituents on the nitrogen proved to decrease the VAP-1/SSAO inhibitory activity. These general structure–activity relationships are well illustrated by some selected data for compounds **40** in Table 1.

Table 1 Substituent effects on the VAP-1/SSAO inhibitory concentrations of hydrazino alcohols **40**.

R^1	R^2	VAP-1/SSAO IC_{50}	MAO IC_{50}	Selectivity[a]
H	Me	0.21 μM	42 μM	200
H	*i*Bu	0.29 μM	15 μM	52
H	cyclohexyl	1.3 μM	1.3 μM	1
H	CH_2-cyclohexyl	2.3 μM	1.1 μM	0.5
H	CH_2Ph	0.04 μM	0.16 μM	4
2′-Cl	Me	0.27 μM	20 μM	85
4′-Cl	Me	0.23 μM	20 μM	87
4′-Cl	*i*Pr	6.3 μM	25 μM	4
4′-Cl	*n*Bu	2.5 μM	2.4 μM	0.96
3′-OMe	Me	0.28 μM	42 μM	150
4′-OMe	Me	0.33 μM	40 μM	121

[a]Selectivity = MAO IC_{50}/VAP-1 IC_{50}.

A CANDIDATE DRUG TO TREAT INFLAMMATORY DISEASE

Some compounds in the current series have shown appropriate chemical, pharmacological, and toxicological profiles and have been tested in vivo in animal models of arthritis. An indane hydrazino alcohol, which significantly reduced clinical symptoms in collagen-induced arthritis in the mouse and adjuvant arthritis in the rat, has been chosen for further development. The results support the view that the VAP-1/SSAO enzyme plays a crucial role in inflammatory diseases and that therapy based on blocking VAP-1/SSAO activity may be clinically valuable.

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